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THE HUMAN REQUIREMENT FOR RIBOFLAVIN.

BORDEN'S REVIEW
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THE HUMAN REQUIREMENT FOR RIBOFLAVIN.

In order to derive a set of allowances representing the best available evidence as to the amounts of the various nutrients required in the average daily diet, the Food and Nutrition Board of the National Research Council began, in 1940, a systematic appraisal of the existing scientific literature on the subject. Critical evaluation of this information combined with opinions solicited from recognized nutrition authorities served as a basis for the formulation of tentative recommendations. After considerable discussion and revision, a final set of values was announced to the public in May, 1941.

Riboflavin Recommendations:

Largely on the basis of data from riboflavin balance studies on male and female adults†, the National Research Council suggested a daily intake of a little over two milligrams for the average woman and almost three milligrams of riboflavin for the average man. The recommended "allowances" for each sex are graded according to degree of activity for adults, and according to specific age groups for infants and children. In the case of adults the correlation between degree of activity and riboflavin intake is designed to take into account the heightened caloric requirement associated with increased activity. As the intake of high calorie foods (particularly of fats and carbohydrates) increases, a corresponding increase is necessitated in the requirement for riboflavin and other B vitamins concerned with the proper metabolism of these foodstuffs. Similar considerations, in addition to the special needs of growth, apply in the case of children. Following are the values recommended as desirable goals for riboflavin intake:

Recommended Dietary Allowances (1)
Food and Nutrition Board, National Research Council

	Riboflavin, Mg.
Man (70 Kg.)	
Sedentary	2.2
Moderately active	2.7
Very active	3.3
Woman (56 Kg.)	
Sedentary	1.8
Moderately active	2.2
Very active	2.7
Pregnancy (latter half)	2.5
Lactation	3.0

	Riboflavin, Mg.
Children up to 12 years:	
Under 1 year	0.6
1-3 years	0.9
4-6 years	1.2
7-9 years	1.5
10-12 years	1.8
Children over 12 years:	
Girls, 13-15 years	2.0
16-20 years	1.8
Boys, 13-15 years	2.4
16-20 years	3.0

EDITOR'S NOTE—This paper is a continuation of "Riboflavin in Human Nutrition" which appeared in the September, 1944 issue of *Borden's Review of Nutrition Research.*

†Described in September, 1944 issue.

Recent Clinical Research:

The recommendations set forth by the National Research Council are intended to serve as a guide for planning adequate nutrition with respect to the individual nutrients listed. In the case of riboflavin, as with other vitamins and the minerals, the quantities given are planned to provide not merely the minima sufficient to protect against actual deficiency disease, but a fair margin of safety above this to insure good nutrition and protection of all body tissues (1). Research nutritionists, however, have been continuing their investigations in an attempt to determine more precisely, if possible, the exact minimum riboflavin requirements of man and to confirm or reevaluate the suggested optimal amounts.

Women: Early in 1943 Williams and associates (2) at the Mayo Clinic reported some interesting facts concerning human riboflavin requirements as indicated by experiments on induced riboflavin deficiency in women. Their subjects were divided into three groups. The first consisted of four women maintained for 288 days on a diet severely restricted in riboflavin, but adequately supplemented with other B vitamins. The second, comprising two subjects, received the same riboflavin-restricted diet over a period of 246 days, but did not receive the crystalline supplements of the other B vitamins. These subjects, therefore, were deficient in all members of the vitamin B complex. A third group of two women served as controls. They were kept on the same standard diet for a period of 288 days, receiving, in addition, crystalline supplements of riboflavin and the other B vitamins so that the diet was not deficient in any essential nutrient. Prior to the experiment, the subjects were fed an adequate diet supplemented with vitamins, so that each began the test with presumably optimum stores of riboflavin.

The first group, subsisting on a diet severely restricted in riboflavin, (0.7 mg. per day) gave no evidence of deficiency. They had good appetites and felt well throughout. Results of all tests were normal. There was no impairment of physiological functions; no disturbances of the eyes, skin or mouth. The only evidence of deficiency observed was a progressive decrease in the excretion of single test doses of riboflavin, indicating tissue depletion. In view of this gradual depletion of tissue stores, it was concluded that clinical evidence of ariboflavinosis would have appeared eventually if the experiment had been allowed to continue. Also, the periodic administration of 2 mg. test doses of riboflavin may have hindered the development of any clinical manifestations.

Data on these subjects, therefore, indicated clearly that a daily intake of 0.7 mg. of riboflavin per day (0.35 mg. per 1000 calories) did not precipitate clinical evidence of riboflavin deficiency, but was insufficient to prevent depletion of tissue stores.

The second group of subjects, restricted to a diet providing only 0.35 mg. riboflavin per 1000 calories and deficient in other vitamins of the B complex developed signs of thiamin deficiency within 100 days. These were promptly cured by thiamin. Clinical evidence of riboflavin deficiency was absent, but the same indications of tissue depletion were shown by urinary excretions as in group I.

Subjects serving as controls for the first two groups received the standard diet supplemented with crystalline riboflavin in amounts ranging from one to three milligrams per day. When they were maintained on a supplement of only one milligram of riboflavin per day no evidence of riboflavin deficiency was observed. Urinary excretion studies of this group when the intake of riboflavin was 1.6 mg. per day or 0.8 mg. per 1000 calories (riboflavin in the standard diet plus 1 mg. supplement) indicated no depletion of tissue stores. For this reason, the authors included additional data from another experiment (3) in which the subjects received only 0.5 mg. of riboflavin per 1000 calories (1 mg. per day). Only slight depletion of tissue stores was noted.

Williams *et al* conclude, therefore, that a daily intake of 0.35 mg. per 1000 calories is insufficient to maintain body stores of riboflavin, and that the minimal requirement is in the neighborhood of 0.5 mg. per 1000 calories but does not exceed 0.8 mg. per 1000 calories.

The low value of 1 mg. riboflavin per day cited by these authors contrasts significantly with Sebrell's (3a) recommendation of approximately 2.5 mg. for the average woman.* Williams *et al* point out, however, that the procedures in their studies differed markedly from those of Sebrell and Butler in that their subjects began the experiments with optimal stores of riboflavin in the tissues, whereas those of Sebrell and Butler had not received extra riboflavin prior to the test and had probably subsisted previously on a diet rather low in the vitamin. The subjects with lower tissue stores would presumably show earlier evidence of riboflavin deficiency. Also, restriction of riboflavin intake was more severe in the experiments of Sebrell and Butler, again predisposing to earlier manifestations of deficiency. Lastly, the periodic administration of liberal test doses of riboflavin

*See Part I of this *Review*, September, 1944.

by Williams *et al* may have inhibited the development of detectable deficiency symptoms. All of these factors would tend to heighten the minimal requirement of the subjects in the experiments of Sebrell.

The Food and Nutrition Board of the National Research Council recommends a daily allowance of 2.2 mg. of riboflavin (0.88 mg. per 1000 calories) for a moderately active woman (see table on page 1). This allowance provides a liberal margin of safety over the tentative minimum suggestion of 1 mg. per day, or even the more liberal suggestion of 1.6 mg. as proposed by Williams *et al* and nearly approaches the high of 2.5 mg. suggested by Sebrell and Butler.

Children: Studies involving children have only recently been undertaken and have been relatively few in number. In 1942 Benson and coworkers (4, 5) reported on the probable thiamin requirement of children as determined by estimation of blood levels and urinary excretion of the vitamin. In June of this year, Oldham and her associates (6) reported results of experiments which extended this work to include data on riboflavin as well. Their studies were limited to two five-year-old boys. Riboflavin excretions of the children were followed as the intakes of the vitamin were progressively increased from very low to quite high levels. These observations were supplemented by determinations of excretions following a one-hour fasting period and of 4-hour and 24-hour recoveries of standard test doses of riboflavin. These tests were designed to throw light on the needs of children of this age for riboflavin and also served as a basis for comparing the efficacy of the different methods for determining nutritional status with regard to riboflavin. Their results are shown in the table on page 5.

As can be noted, the average daily excretions of both subjects decreased progressively at the 250 and 275 mcgm. levels of intake and increased progressively at the 550 mcgm. level. This was interpreted as evidence of a gradual depletion of tissue riboflavin at the lower level and a gradual rebuilding of tissue stores at the higher level. A more or less constant excretion level was reached at intakes of 700 and 725 mcgm. (0.53 mg. per 1000 calories). This lowest level at which the daily excretions neither increased or decreased was considered the minimal requirement for the children studied and, probably, for other children of this age and activity. When the intake was at this level, the 1-hour fasting excretions were 9 mcgm. and the 4-hour returns of test doses approximately 20%. Since there was good correlation of the 1-hour fasting excretions and the 4 and 24-hour test

Riboflavin excretions of preschool children on different intakes (6)

	SUBJECT 1					SUBJECT 2				
	Av. Daily Intake		Riboflavin Excretion				Av. Daily Intake		Riboflavin Excretion		
Periods (days)	Calories	Riboflavin mcgm.	Av. per 24 hr. in each period mcgm.	4-hr. after test dose mcgm.	Av. per 1 hr. fasting[1] mcgm.	Periods (days)	Calories	Riboflavin mcgm.	Av. per 24 hr. in each period mcgm.	4-hr. after test dose mcgm.	Av. per 1 hr. fasting[1] mcgm.
1-6	1290	250	50, 40, 30, 20, 20, 20	140	1	1-4	1520	275	50, 20, 20, 30	80	2
7-12	1390	550	30, 40, 40, 40, 70, 70	160	4	5-10	1570	550	30, 30, 50, 60, 70, 80	150	5
13-18	1310	700	130, 140, 130, 140, 110, 50	240	9	11-16	1380	725	110, 90, 100, 90, 100, 140	220	9
19-26[2]	1450	850	100, 70, 470, 200 180, 180, 120, 90	100	3	17-26[2]	1410	875	170, 160, 210, 150, 340, 440, 300, 170, 100, 110	140	7
27-31	1370	1050	90, 230 230, 200, 210	110	13	27-31	1690	1075	160, 140, 220, 250, 270	280	14
32-36	1540	1450	320, 340 330, 400, 170	420	23	32-36	1520	1425	470, 430, 320, 490 440	360	23
37	....	....	5080	...	..	37	....	....	5050	...	..
38	....	....	990	440	35	38	....	....	830	440	25

[1]The 1-hour fasting values represent the average of two or more determinations, carried out on successive days.

[2]During periods 21 and 22 both subjects had acute upper respiratory infections.

dose returns with each other and with the intake, it is suggested, on the basis of the limited data available, that determination of 1-hour fasting excretions or returns of 4-hour test doses can be used successfully as measurements of satisfactory nutritional status (using the criteria cited above) with respect to riboflavin.

The riboflavin intake suggested by Oldham *et al* for children in the 4–6 age group is approximately 0.5 mg. per 1000 calories. This figure, interestingly enough, agrees with that of Williams *et al* (2) for adults. The recommended allowance of the National Research Council for children in this age group is 0.75 mg. per 1000, or about one-third more, thus supplying an adequate margin of safety.

Men: Early this year, Keys and associates (7) at the University of Minnesota conducted an exhaustive series of studies on the effect of riboflavin restriction on various physiological and biochemical functions in young men. These studies were somewhat similar to the earlier investigations of Sebrell and Williams and their respective coworkers on female subjects. Keys *et al* increased the scope of these investigations by studying variations in physical performance and other objective functional tests.

The tests were conducted on six normal young men who were placed for more than five months on an intake of riboflavin restricted to about one-third the recommended allowance of the National Research Council. The actual riboflavin content of the diet averaged 0.99 mg. per day (0.3 mg. per 1000 calories), or slightly below the suggested minimum of 1 mg. (0.5 mg. per 1000 calories) of Williams and coworkers†. A wide variety of standardized tests was employed in an attempt to measure quantitatively the effect of the restricted diet on biological, physical and physiological functions.*

Except for a decrease in the urinary output of riboflavin, no perceptible or significant changes were noted. Responses to biological tests were essentially negative and no impairment in work capacity was observed. The authors conclude that "normal young men suffer no physiological handicap from subsistence for at least five months on a diet providing 0.3 mg. of riboflavin per 1000 calories."

A few months later, the investigators at Minnesota (8) published results obtained from another series of experiments in which the subjects, eight normal young men, were placed on diets restricted not only in riboflavin, but in the three B vitamins—thiamin, riboflavin, niacin. Here again, no effect other than a drop in riboflavin excretion could be observed in any of the subjects. These results appear to be in direct conflict with the earlier work of Johnson *et al* (9) who reported marked physical and psychological deterioration in only a few days of hard work on diets low in the B vitamins. Johnson *et al,* in an addendum to the paper by Keys and coworkers (8), attempt to reconcile these differences on the basis of variations in the experimental conditions employed and other factors.

The recent demonstration by Najjar and Holt (15) of the biosynthesis of thiamin in man by bacterial action in the intestine introduces another factor which must be considered in any estimation of B vitamin requirements. That riboflavin may also be synthesized in man by intestinal bacteria is a further possibility. Individual variations in the ability to synthesize vitamins in the intestinal tract depend upon many factors. These include, among others, the nature of the diet as a whole, environmental conditions influencing the type and activity of bacterial flora in the intestine, and possibly, therapy with sulfonamide drugs having bacteriostatic effects. The concept of

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†Total calories varied in each group.

*The tests included 60 minutes of hard work on a treadmill, an anaerobic work test, glucose tolerance, muscle power and psycho-motor tests. Observations included pulse rates under varying conditions, metabolism, blood levels of lactic acid, pyruvic acid, glucose and hemoglobin, and urinary excretion of riboflavin with and without "saturation" test doses. Clinical examination included slit lamp and other special observations.

human intestinal synthesis adds another facet to the complex picture of vitamin requirements and may very well offer one explanation for the apparent discrepancies reported in the literature.

Riboflavin-Thiamin Interrelationships:

One of the factors complicating the picture of riboflavin deficiency is the effect of other components of the diet on the utilization and retention of riboflavin. Very little precise information is available as to the extent or nature of the interrelationships involved, but there are definite indications that some such effect does exist. Perhaps the most significant, and certainly the most controversial, of such interrelationships is the one between riboflavin and thiamin. Sure and Ford (10) have reported an increase in the excretion of riboflavin in the case of experimental animals rendered deficient in thiamin. They postulate that insufficient thiamin interferes with the metabolism and retention of riboflavin, so that large amounts of the latter are lost in thiamin deficiency. These findings have been disputed by Ferrebee and Weissman (11), but Sure (12) regards the data upon which their conclusions are drawn as insufficient and suggests specific clinical studies with human beings before the problem can be resolved.

That some specific interrelationship between these two vitamins does obtain, however, has been pointed out by other workers. Supplee and associates (13) observed that the concentration of riboflavin in the liver of rats increased temporarily following the ingestion of food and that this mobilization of riboflavin to the liver was interfered with in thiamine- or pantothenic acid-deficient animals. The actual concentration of riboflavin in the liver, on the other hand, was found by Singher *et al* (14) to be significantly increased in thiamin-deficient animals. Conversely, thiamin concentration in the liver rose with riboflavin deficiency. Deficiencies of other B vitamins or of vitamin A had no appreciable effect on the concentration of either riboflavin or thiamin in the liver. These facts have been interpreted as evidence of a direct interdependence of thiamin and riboflavin.

These studies lead to the undisputable conclusion that significant interrelationships do exist between thiamin and riboflavin. The mechanisms of these relationships and the specific influences mutually exerted are still in the process of clarification and it is to be hoped that future research will soon elucidate these problems. In the meantime, it should be borne in mind that vitamin interrelationships must be recognized and remain to be reckoned with as important factors in the estimation of individual vitamin requirements.

Evaluation of Existing Data:

Because of the wide variations in experimental methods and because of the relative uncertainty as to what constitute the exact criteria of human riboflavin deficiency, it is almost impossible to evaluate accurately the conclusions reported. Discrepancies between the work of Sebrell and of Williams on females have already been discussed. The recent work on males by Keys and others differs from both of the former workers in many ways. The female subjects, for example, were kept on considerably lower caloric intakes and were quite sedentary. On a calorie-vitamin ratio basis, the male subjects received more riboflavin than did Sebrell's and less than Williams'. Further, prior to the experiment, Sebrell's subjects were somewhat malnourished, Williams' were thoroughly fortified with riboflavin, and Keys' were on an average, presumably adequate diet. Other minor factors were undoubtedly also involved.

One fact remains clear. Although there is disagreement as to whether one or three milligrams more nearly approaches the requirement of riboflavin for adults, animal experiments indicate the importance of optimal rather than minimal intakes of this vitamin.*

Long-term subsistence on minimum levels of riboflavin intake extending over periods of years, as contrasted with experimental periods of months, may so deplete tissue stores as to increase predisposition to ariboflavinosis. This again suggests the advisability of allowing a liberal "margin of safety" when setting riboflavin standards.

Prevalence of Riboflavin Deficiency: The widespread occurrence of riboflavin deficiency in the United States confirms the importance of establishing liberal standards for the intake of riboflavin. Some indication of the extent of riboflavin deficiency can be gathered from the review on nutritional deficiencies in the United States recently published by the National Research Council (16) and abstracted in the May, 1944 issue of this *Review*.

Subsequent to the publication of this report, several additional surveys have been conducted in various localities with representative age and income groups. Among these have been the studies of Winters and Leslie (17, 18) on women on low income and moderate income levels. While in general the diets of women in the moderate income group reflected a greater choice of nutritious foods, mild deficiencies in dietary intake still existed. In the low income group, riboflavin intakes were approximately one-third the recommended allowances;

*Sherman and Ellis—discussed in Part I of this *Review*, September, 1944.

in the moderate income group the riboflavin intakes were four-fifths of the recommended allowances. The more nearly adequate intake of riboflavin was achieved by a large consumption of milk.

A few months ago, Feder, Lewis and Alden (19) reported fairly widespread riboflavin deficiency among several typical groups. They found that 25 per cent of a group of medical students were deficient in riboflavin, as evidenced by excretion studies. In a group of white draftees of approximately the same age, but differing economic status, about 30% were found to be deficient, while 50% of a corresponding group of colored draftees showed evidence of riboflavin deficiency. A group of hospital patients showed still higher percentages of riboflavin deficiency, but this was more or less expected because of the highly selected nature of the group. Of persons selected at random in a rural area, 65 per cent showed a deficiency indicating, in the opinion of the authors, that a low intake of riboflavin was widespread throughout the general population in the area (Georgia) studied.

Most recent clinical and dietary studies, therefore, although limited in scope, confirm the findings of the National Research Council in 1943 (16) of the widespread occurrence of riboflavin deficiency.

Food Sources of Riboflavin:

"In practice, the actual importance of any given food as a dietary source of riboflavin depends of course not only on the concentration of the substance in the food, but also in the place (or quantitative prominence) of the food in the dietary or food supply," state Sherman and Lanford (20). On this basis they list milk as the most significant source of riboflavin in the diet, especially for children (see table).

Relative Prominence of Certain Types of Food (in Dietaries Costing from $2.38 to $3 Weekly per Food-Cost Unit)*

Food or Food Group	Per Cent of Food Money Allocated	Per Cent Riboflavin Contributed by Each Group
Meats and fish	25.5	32.2
Eggs	5.0	5.3
Milk, cheese, ice cream	12.1	34.2
Butter, cream	7.6	0.4
Other fats	3.0	1.1
Breadstuffs, cereals, bakery products	17.6	5.7
Sugar, sweets	4.2	...
Potatoes, sweet potatoes	1.5	7.2
Dried legumes, nuts	1.8	0.8
Tomatoes	1.5	0.6
Citrus fruits	3.2	3.3
Green and yellow vegetables	3.9	3.7
Other vegetables	3.4	1.2
Other fruits	3.9	3.9

*Adapted from Stiebeling, 1936 (21).

Liver is a very highly concentrated source of riboflavin, but its restricted occurrence in the diet is such as to minimize its value as a

source of the vitamin. Yeast, likewise an excellent source of riboflavin, does not constitute a significant part of the diet. Other good sources of riboflavin include beside milk, cheese, eggs (especially egg yolk), ice cream, kidney, beef and chicken muscle, green leafy vegetables (kale, spinach), and wheat germ. Bread fortified with skim milk solids provides another good supplementary source of riboflavin.

Conclusion:

Experimental as well as clinical evidence of the past decade has established the primary importance of riboflavin in human dietaries. A distinct disease entity, ariboflavinosis, has been described wherein characteristic deficiency symptoms appear in the tissues of the eyes, mouth and skin. These symptoms respond dramatically to riboflavin therapy. In cases of non-nutritional origin, similar symptoms may appear—particularly in the eye—but these must be distinguished from the manifestations of ariboflavinosis and treated accordingly.

Evidence of fairly widespread riboflavin deficiency in the population of the United States is apparent through the recognition of clinical signs of ariboflavinosis, through the extensive investigation of the diets of various population groups, and through more subtle methods such as the study of riboflavin excretion levels. Much controversy exists in the interpretation of these different forms of evidence, particularly with respect to that derived from excretion studies. When, however, the differences in experimental conditions, in the state of tissue saturation of individuals, in types of experimental diets, in activities and environmental surroundings of subjects and other factors are taken into account, a more hopeful outlook in reconciling conflicting data becomes possible.

Despite the conflicting data, it has been possible to suggest approximate levels of riboflavin intake for guidance in planning practical dietaries. In this connection, it is interesting that in 1943, two years after its original promulgation of riboflavin allowances, the National Research Council did not deem it necessary to change its recommendations even in the light of newer data reported.

The newly recognized possibility of the human biosynthesis of vitamins in the intestinal tract and the growing awareness of the interrelationships existing between individual vitamins are opening up new avenues of investigation which may soon lead to more precise information with regard to quantitative human requirements for riboflavin (and other vitamins), as well as to a fuller understanding of the mode of action of these vitamins.

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NEWS DIGEST

TRYPTOPHANE – A possible relationship between deficiency of the amino acid, tryptophane, and reproductive failure is indicated by recent animal studies. Normal adult male and female rats were fed a tryptophane-deficient diet after mating. All the females on the deficient diet failed to produce litters while those on the control diet reproduced normally. In a group of deficient animals, embryos of normal size were found in females sacrificed on the ninth day of gestation, but those maintained till fourteenth day showed almost complete resorption of the embryos. It is concluded that tryptophane is essential for normal gestation in the rat and that the available stores of this amino acid are depleted in the adult female after ten days on a tryptophane-deficient diet. *Science 97,* 312 (1943).

VITAMINS IN SWEAT – Healthy young male volunteers receiving a normal diet with and without vitamin supplementation were studied for vitamin content of both thermal sweat and sweat induced by exercise. No ascorbic acid was found in any sweat samples, although dehydroascorbic acid was excreted at the rate of about 0.2 mg. per hour. The amounts of thiamin, riboflavin and pantothenic acid found in thermal sweat were similar to those for sweat induced by exercise. Values were not greatly influenced by the amount of vitamin ingested although this influenced the urinary excretion. Thiamin losses in sweat averaged between 0.5 and 1.4 micrograms per hour, respectively for the undosed and dosed subjects; riboflavin, 10 micrograms for both; and pantothenic acid, 24 to 50 micrograms for the two groups. *J. Biol. Chem. 148,* 359 (1943).

CANNED FRUITS AND VEGETABLES–Results of recent research investigations reveal that the chief nutritive values of canned fruits and vegetables are represented by their vitamin and mineral contents. The energy, carbohydrate, protein and fats derived from these processed foods are not of significant importance in the diet. Canned citrus fruits and tomato products are among the more valuable dietary sources of ascorbic acid. The provitamins A contained in canned leafy green and yellow vegetables constitute a major source of vitamin A in our diets. More concentrated food sources of the B vitamins and minerals are not found among the fruits and vegetables either fresh or canned. However, many canned fruits and vegetables contain important quantities of niacin, thiamin, riboflavin, calcium, phosphorus, and iron, nutrients which many diets supply in sub-optimal quantities. *Am. J. Pub. Health 34,* 597 (1944).

GERMINATION AND VITAMINS –Dried peas, soybeans, and other legumes have been investigated in relation to their generation of ascorbic acid, thiamine, riboflavin, and nicotinic acid following germination. It has been determined that, generally speaking, such sprouted legumes are an excellent source of ascorbic acid, fair as a source of riboflavin and nicotinic acid, and poor for thiamine. The various varieties of peas were more productive of the vitamins concerned than were soybeans, which, however, were of considerable merit and markedly superior to the other types of beans investigated. A temperature effect was determined, indicating that both germination and the generation of vitamins proceeded at a faster rate at the higher temperature range of 68° to 97° F. than at 64° to 75° F. The applicability to military conditions of this method of producing an accessory supply of water soluble vitamins has been suggested. *J. Nutr. 28,* 63 (1944).